ADVANCES IN RODENT RESEARCH MISSIONS ON THE INTERNATIONAL SPACE STATION

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BACKGROUND

- Rodent Research is important for biomedical discovery on Earth and in space.
- National Research Council's Decadal Survey emphasized the importance of expanding NASA's life sciences research for long-duration rodent experiments on ISS
- New hardware, operations, and science capabilities developed at NASA ARC to support rodent missions in space

Three Rodent missions flown to date

1. Rodent Research-1: launched on 9/21/14
   - 20 female adult mice (10 for NASA's validation mission and 10 for the National Lab's science experiment)
   - Mission duration: 33 days (NASA's Validation) and 16-17 days (Novartis) on the ISS
2. Rodent Research-2: launched on 4/14/15
   - 20 female adult mice for Novartis/US National Laboratory
   - Mission duration: various time points – up to 8 weeks on the ISS
3. Rodent Research-3: launched on 4/8/16
   - 20 female adult mice for Eli Lilly/US National Laboratory
   - Mission duration: 6 weeks on the ISS

Rodent Research-1

RR-1 Objectives

NASA Validation flight

1. Validate hardware and on-orbit operations to provide reliable housing for the mice for long duration missions (> 1 month)

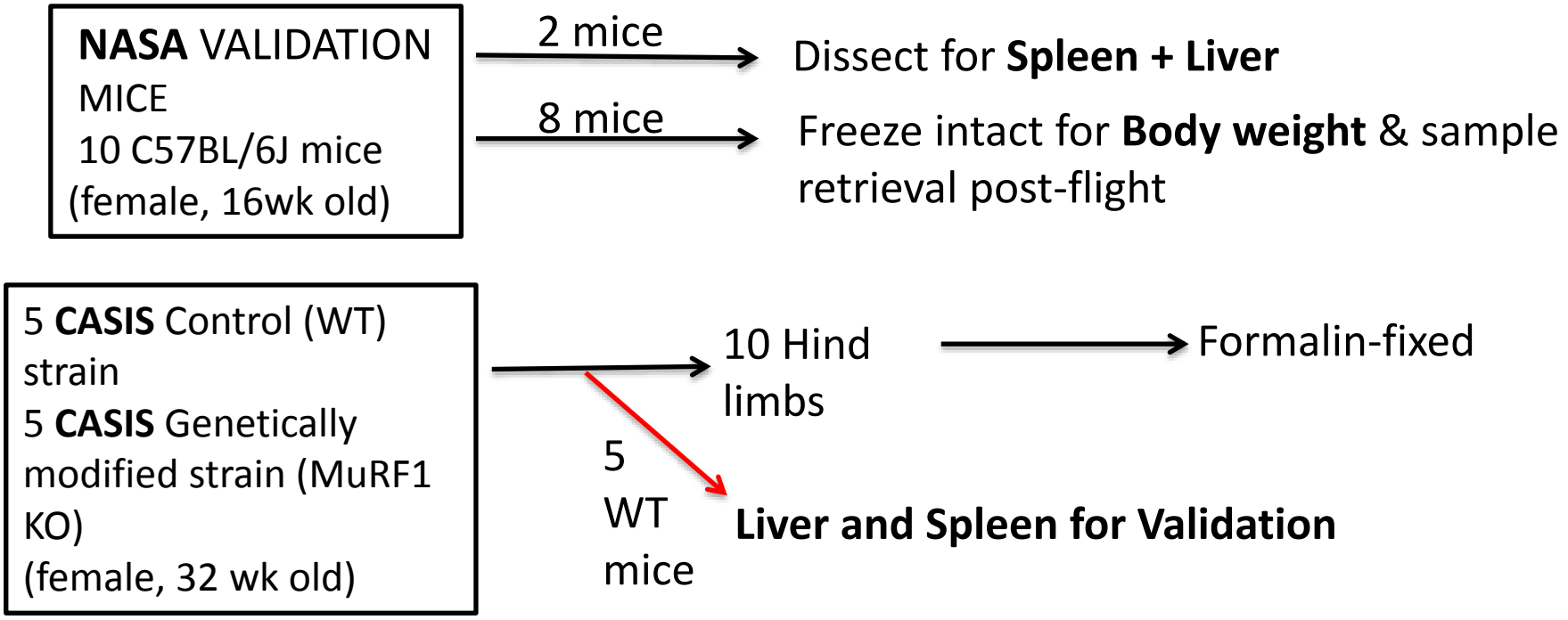
1. ISS's capability to perform and support scientific activity
   - Animal health and behavior
   - Sample retrieval including dissection and tissue preservation

Novartis experiment

1. Validate ability to conduct commercial rodent research on the ISS

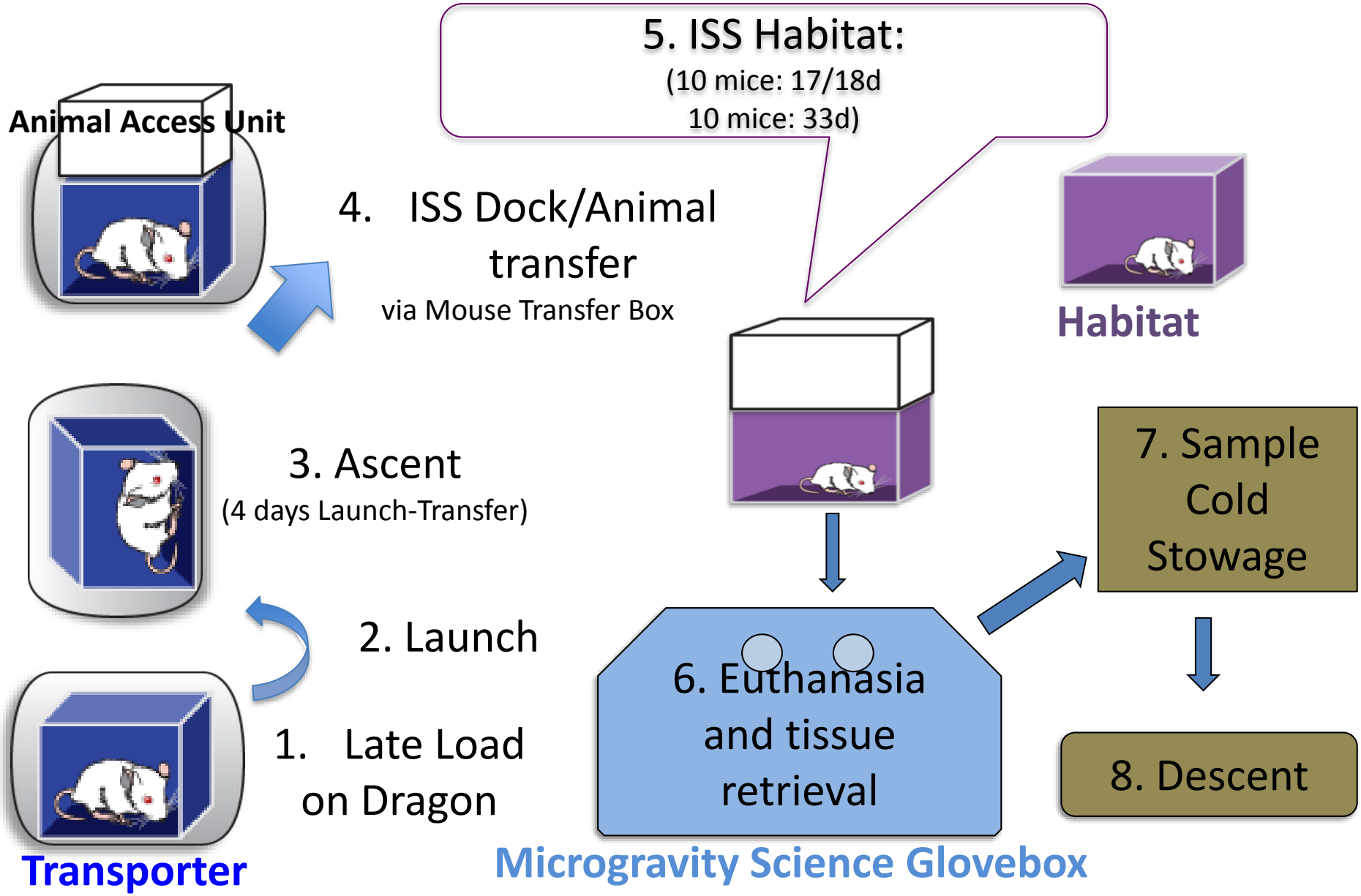
1. Evaluate a specific transgenic strain to prove the mechanisms of spaceflight-induced muscle wasting

RR1 Experimental Design

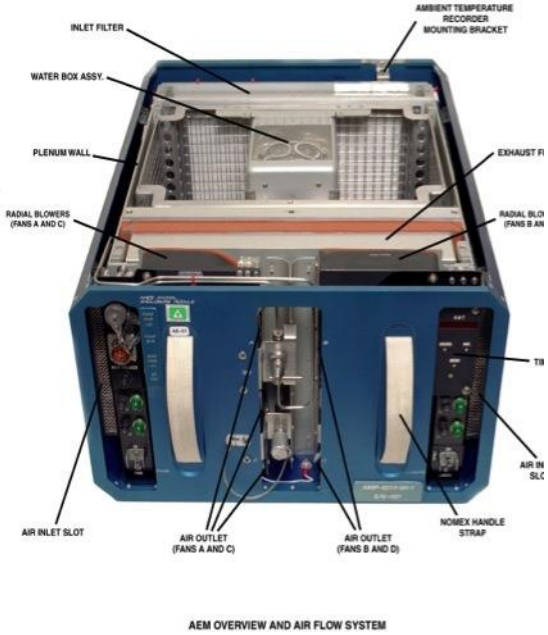


Liver: fast frozen: RNA analysis and enzyme activity measurement
Spleen: preserved in RNAlater: RNA analysis
Daily video monitoring and post-flight behavior analysis
Measurements of body weights and organ weights to assess stress responses

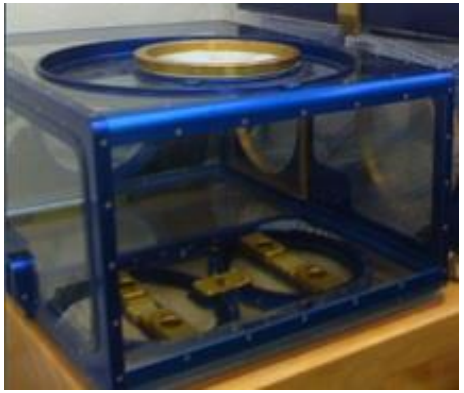
RR1: Concept of Operations



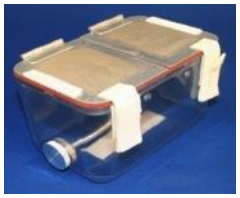
Rodent Hardware



Transporter



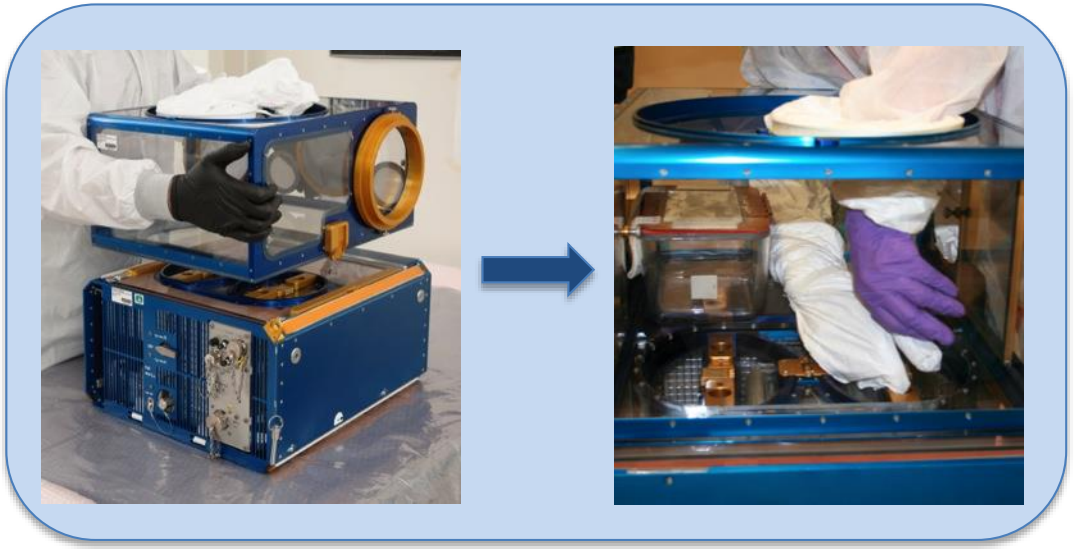
Animal Access Unit



Mouse Transfer Box

Kits (many)

Habitat



Mice behavior on ISS: Validation mice

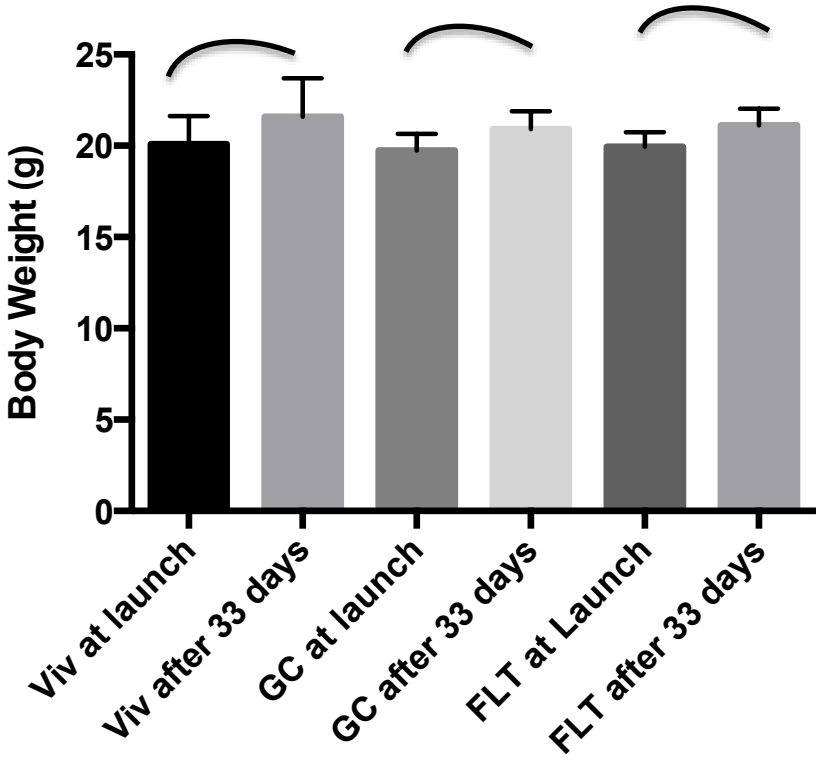
Qualitative and Quantitative analyses made using daily health check videos

- Upon initial introduction into the Habitat, mice actively explored the compartments
- Mice were observed eating, drinking, grooming and socially interacting while in the Habitats
    - No major differences in numbers of mice observed feeding across spaceflight conditions
    - FLT mice initially spent more time self-grooming relative to GCs, but the occurrence of grooming was similar in FLT and GC mice throughout the remainder of the flight
- Mice propelled themselves freely and actively throughout the Habitat using their forelimbs
    - Mostly by 'pulling' along cage grate with their forelimbs or by 'floating' from one location to another; later on orbit, began to use hindlimbs also to move along cage walls.
- As time went on, the mice moved more quickly around the compartment, moving with ease through open spaces and anchoring themselves using tails and/or paws
- "Race-tracking" behavior observed exclusively in FLT mice during the dark cycle

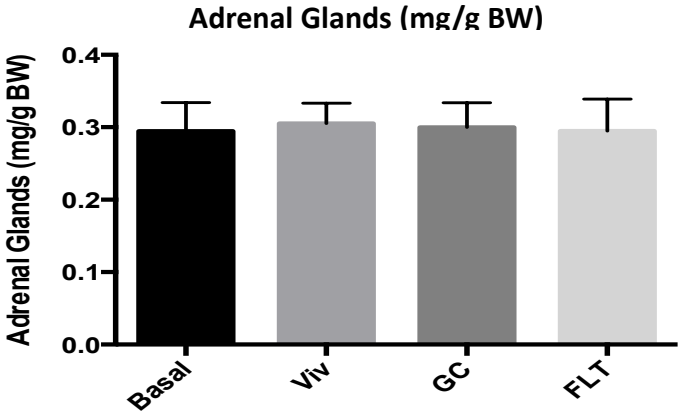
Video clip

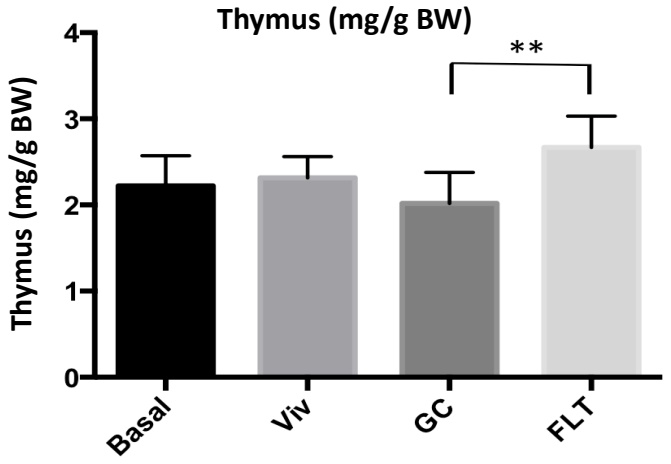
Summary of RR1 findings

Body Weights: no difference between Flight and Ground Controls



No signs of chronic stress based on the tissue weights





Summary of RR1 findings (cont'd)

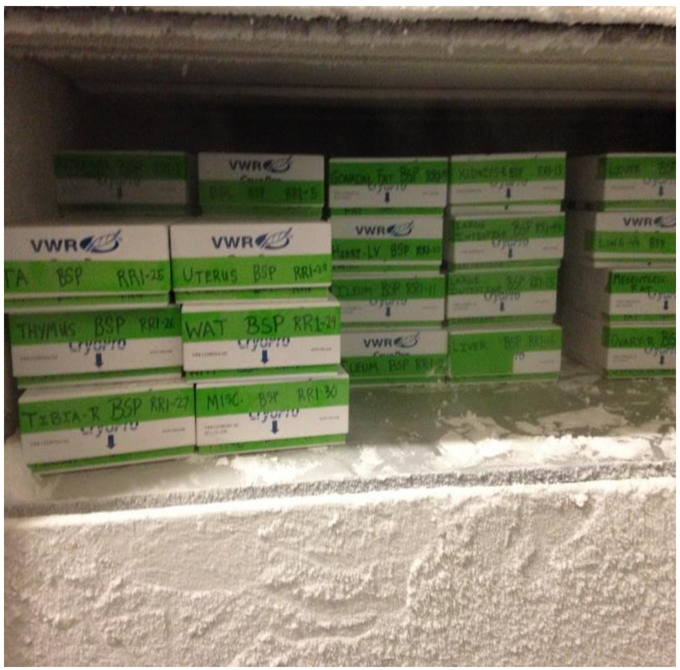
- High quality samples suitable for applying cutting edge molecular biological methods were recovered
  - Livers and spleens dissected on orbit post-euthanasia (RIN≥8)
  - Livers dissected from frozen carcasses after return to Earth (RIN≥8)
  - Liver samples collected from NASA's validation mice and Novartis mice were provided to GeneLab and analyzed for transcriptomics, epigenetics and proteomics
    - the data are currently publically available:

      https://genelab-data.ndc.nasa.gov/genelab/search_studies/?q=RR1

- Activity of liver enzymes (catalase, glutathione reductase and glyceraldehyde-3-phosphate dehydrogenase) were preserved in samples collected from frozen carcasses

Expanding science return from RR1 through Biospecimen Sharing Program (BSP)

- RR science team recovered 32 tissues from 40 RR1 Validation mice including flight, ground controls, baseline and vivarium controls, yielding total of 3280 vials of tissues
- Additional 7 tissues were recovered from second thaw of the carcasses



Ames Life Science Data Archive (LSDA) Biospecimen Storage Facility

1) BSP tissues were distributed to the scientific community through the Ames Life Science Data Archive (LSDA)
2) Select samples were provided to Russian research colleagues at the Institute for Biomedical Problems (IBMP)
3) NASA GeneLab project
   - Various tissues were provided for “omics” analyses

RR1 Conclusion

- Hardware performed as expected and operations completed successfully on orbit for animal transfer, euthanasia, dissection and sample preservation
- Established baseline mission systems and biological database to help guide future rodent research on ISS
- Provided tissue samples for the NASA Space Biology-Biospecimen Sharing Program and the GeneLab's omics (transcriptomic, epigenomic and proteomic) analyses

NASA ARC collaborates with commercial partners and the U.S. National Laboratory through CASIS

Rodent Research-2

Rodent Research-3

RR-2 and RR-3 Achievements

	RR-2 (Novartis)	RR-3 (Eli Lilly)
Mice	20 C57BL/6 Female	20 BALB/c Female
New capabilities	Bone densitometry scan	Bone densitometry scan with Anesthesia Recovery System (ARS) Grip strength assessment
Hardware	Standard RR Hardware	Standard RR Hardware Bone Densitometer, ARS, Grip Strength Meter
On-orbit Dissection	Blood draw and separation Hindlimb and eye fixation Hindlimb dissection and freezing Carcass freezing	Blood draw and separation Hindlimb fixation Carcass freezing
Days on ISS	Various (up to 54 days)	6 weeks

New capabilities developed for use on subsequent missions since RR-1

- RR-2 and RR-3 missions:
  - Bone densitometry scans
  - Grip strength measurements
  - Recovery from anesthesia

- RR-4:
  - Male mice
  - Increase the number of mice from 20 to 40

- RR-5: Live animal return

- JRR-1 (Joint Rodent Research-1): Joint mission between NASA SLPS and Russian scientists

Rodent Research-4

- Objectives: to study bone healing in microgravity
- First mission using male mice
- 40 C57BL/6J mice will be flown on SpaceX 10
- On-orbit dissections:
  - Blood collection
  - Hindlimb dissection/fixation
  - Carcass freezing

Conclusion

- NASA ARC Rodent Research Project continues to:
  - expand capabilities to perform long-duration missions on the ISS
  - maximize science return through Biospecimen Sharing Program
  - contribute to understanding of the effects of microgravity on human health as strongly emphasized in the NRC's 2011 Decadal Survey, "Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era"

Acknowledgements

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Rodent Habitat: RR Scientific subject matter experts

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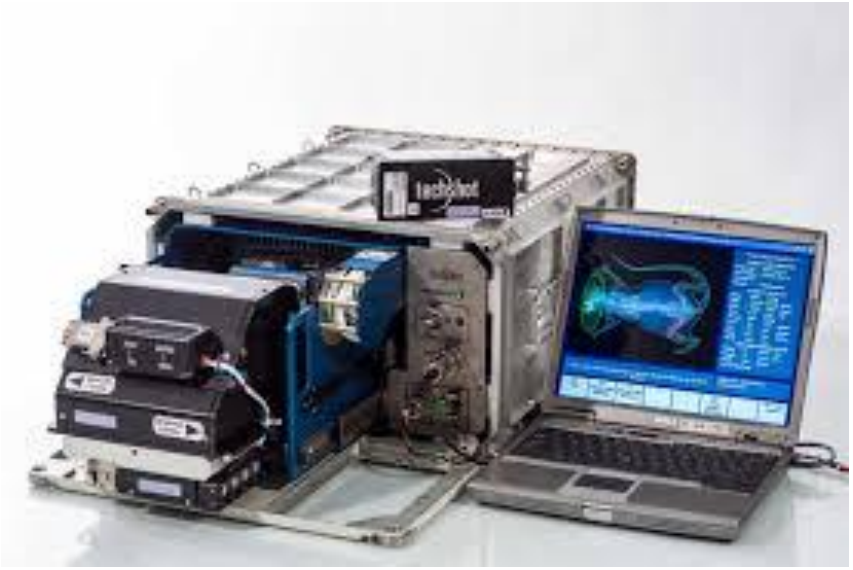
EXTRA

Bone Densitometer (RR-2 and RR-3)

- Quantitative measures of bone and muscle loss in mice during orbital space
- Dual Energy X-ray Absorptiometry (DEXA)
  - X-ray System for Measuring Bone Density
  - Measures soft-tissue density, lean/fat ratio and total animal mass (i.e. weighing mice in space)
- Fast imaging in less than 5 minutes, allows faster access to important data and safer on animals



Anesthesia Recovery System

- Mice become rapidly hypothermic due to anesthesia
- Supplemental heating provided to support recovery
- Consists of a heated block holds up to 5 tubes at a time
- Each tube (clear polycarbonate, 6” long x 1.5” in diameter) holds a single mouse and vented on both ends to ensure oxygen and carbon dioxide circulation
- Used for the first time for RR3 mission

Grip Strength Meter (RR-3)

- Records the peak force required to remove the mouse from the grid
- To be repeated up to 4 times for each mouse and averaged of 3 or 4 peak force measurements be used as the grip strength per mouse